Napping Reverses the Salivary Interleukin-6 and Urinary Norepinephrine Changes Induced by Sleep Restriction

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Context: Neuroendocrine and immune stresses imposed by chronic sleep restriction are known to be involved in the harmful cardiovascular effects associated with poor sleep.

Objectives: Despite a well-known beneficial effect of napping on alertness, its effects on neuroendocrine stress and immune responses after sleep restriction are largely unknown.

Design: This study was a strictly controlled (sleep-wake status, light environment, caloric intake), crossover, randomized design in continuously polysomnography-monitored subjects.

Setting: The study was conducted in a laboratory-based study.

Participants: The subjects were 11 healthy young men.

Intervention: We investigated the effects on neuroendocrine and immune biomarkers of a night of sleep restricted to 2 h followed by a day without naps or with 30 minute morning and afternoon naps, both conditions followed by an ad libitum recovery night starting at 20:00.

Main Outcome Measures: Salivary interleukin-6 and urinary catecholamines were assessed throughout the daytime study periods.

Results: The increase in norepinephrine values seen at the end of the afternoon after the sleep-restricted night was not present when the subjects had the opportunity to take naps. Interleukin-6 changes observed after sleep deprivation were also normalized after napping. During the recovery day in the no-nap condition, there were increased levels of afternoon epinephrine and dopamine, which was not the case in the nap condition. A recovery night after napping was associated with a reduced amount of slow-wave sleep compared to after the no-nap condition.

Conclusions: Our data suggest that napping has stress-releasing and immune effects. Napping could be easily applied in real settings as a countermeasure to the detrimental health consequences of sleep debt. **(*J Clin Endocrinol Metab* 100: E416–E426, 2015)**

Neuroendocrine and immune functions are regulated by circadian pacemakers and homeostatic sleep systems through hormones and neural connections. It is commonly thought that the most important effect of nighttime sleep loss is daytime sleepiness, resulting in cognitive impairment and an increased incidence of errors and

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Abbreviations: BMI, body mass index; CNS, central nervous system; EIA, enzyme immunoassay; HPA, hypothalamic-pituitary-adrenal; SWS, slow-wave sleep.

transport accidents. However, sleep deprivation is also increasingly described as inducing an imbalance in metabolic, hormonal, and immune homeostasis, with important public-health relevance (1–4).

The physiological mechanisms linking sleep deprivation and immune and inflammatory changes and leading to the progression of cardiovascular pathogenesis are poorly understood. Under normal physiological conditions, there are low concentrations of cytokines in the blood, except for interleukin (IL)-6, which is a cytokine with hormone-like actions. Several studies have reported diurnal variations in various cytokines, including IL-6, IL-12 or tumor necrosis factor (TNF)-α, as well as in the leukocyte subset cells that are responsible for their production (5, 6). The change in inflammatory cytokines, such as IL-6, possibly induced by the enhanced release of the stress mediators, cortisol and catecholamine, is one potential pathway occurring in postsleep deprivation (3). Whether these immune and inflammatory alterations are associated with activation of a nonspecific immune response or with the neuroendocrine stress response that occurs, postsleep deprivation is still under debate. When adaptive physiological processes and/or sleep recovery are unable to correct the development of these pathophysiological pathways, epidemiological data indicate that short sleep durations are associated with a gradual increase in cardiovascular and cancer risk (3, 7–10).

Napping countermeasures may reduce these deleterious health effects by improving the recovery of the neuroendocrine and immune systems. A population-based study investigated 23 681 individuals and reported that midday napping in healthy working men was inversely associated with coronary mortality after controlling for potential confounders (11). Previous laboratory studies mainly assessed the countermeasure aspect of napping on vigilance, and to a lesser extent its effects on neuroendocrine stress and immune biomarkers (12–14). Napping (mainly acting on the homeostatic process) is an effective strategy to combat fatigue and sleepiness during long working hours, especially in young people who are more sensitive to sleep loss and show a greater homeostatic pressure postsleep deprivation than do older subjects (15). A short nap, especially during the postnoon nap zone, has been shown to restore alertness and promote performance and memory processing without the inconvenience of sleep inertia associated with longer naps (16, 17). While some data reported that short naps (~20 minutes) could improve mood status in healthy subjects (18, 19), in a more clinical view, napping could exert as well negative effects in the specific case of sleep-deprived depressed patients. Indeed, sleep deprivation for a single night has previously been described to exert transient clinical beneficial effects on mood status the following day in many depressed patients (20). Short naps and especially morning naps the day following the night of sleep deprivation have been described to induce depression relapses in depressed patient responders to sleep deprivation (21, 22). However, the effects on neuroendocrine stress and immune responses of a short nap following sleep restriction in healthy subjects are largely unknown. We therefore assessed the effects of napping on neuroendocrine and immune biomarkers. To achieve this, we used noninvasive measurement methods, sampling saliva and urine. The choice of measurement technique is important when assessing biomarkers that are potentially sensitive to the sleep debt status of the subject and this approach may represent a potential tool for future investigations in larger populations affected by sleep loss.

Materials and Methods

Ethical issues

The study was conducted according to French regulations on human research including agreements from the Hotel-Dieu Hospital Ethics Committee (CPP-I Ile de France 1) and signed consent from participants who received financial compensation. The study described in this protocol was conducted in accordance with the current version of the declaration of Helsinki and ICH guidelines for Good Clinical Practice.

Subjects

Eleven healthy, nonsmoking men aged 25–32 years (mean ± sem 27 ± 1.6 years), not regular nappers, with a body mass index (BMI) between 19 and 25 participated in the experiment. All volunteers had no sleep complaints, slept regular nights of 7–9 h, and were intermediate chronotype, as indicated by sleep and chronotype questionnaires and one adaptation night of polysomnography monitoring. Each subject was in good health as reported by an initial medical examination with no depression, anxiety or emotional distress detected using the Hospital Anxiety and Depression Scale (23). Subjects were recruited by advertising the study in the Hospital and on the university campus.

Experimental design

The experimental design included two 3-day sessions that the volunteers performed in random order (see Supplemental Figure 1). Prior to their admission to the sleep laboratory, the participants completed one week of regular sleep-wake behavior with 8 h of sleep (in bed from 00:00 to 08:00) documented by actigraphic recordings and sleep diaries. During the study, the volunteers remained in the sleep laboratory under continuous supervision by the investigators. Throughout the experiment, the light environment and physical activity levels were controlled using an ambulatory actigraphy device, recording luminous flux, and irradiance (Philips Respironics®). Subjects received controlled meals of a maximum of 2500 cal/d with a balanced proportion of nutrients (protein, fat, carbohydrates). Intake of any medication, alcohol, or xanthine derivatives (coffee, tea, chocolate, and cola) was prohibited throughout the study period.

Controlled drinks and snacks were available during the sleep-restricted nights until 00:00. Staff members remained with the volunteers during the period of restricted sleep and the volunteers were provided with films and games during the night of sleep restriction.

In the "sleep restriction" session, the volunteers were sleep restricted to 2 h for 1 night (in bed from 02:00 to 04:00) after 1 baseline night of 8 h of sleep (in bed from 00:00 to 08:00). After the sleep restricted night, volunteers underwent an ad libitum recovery night sleeping from 20:00 until they woke up.

In the "sleep restriction + nap" session, the same volunteers repeated the protocol described above but had two 30-minute naps starting at 09:30 and 15:30 the day following the sleep restricted night.

Sleep monitoring and analyzes

Continuous ambulatory polysomnography recordings were performed throughout the study using an ambulatory device [Dream®, Medatec with the following EEG derivations (C4/A1, C3/A2, O2/A1, O1/A2 F4/A1, F3/A2)] to monitor sleep and wakefulness, and to check the compliance of the subjects to the imposed sleep-wake schedule. Volunteers were free to move within the unit carrying this ambulatory device. Sleep recordings were scored visually in all subjects according to the 2007 American Academy of Sleep Medicine criteria (24).

Assays and measurements

Saliva samples were taken every 2 h, including immediately after the naps, and urine was sampled every in 3 h-period during the awake period from 10:00 to 19:00. The sampling started from the time of awakening for the ad libitum recovery sleep condition. Saliva samples for analysis were immediately placed on ice and then stored at −80°C until assayed. For IL-6 measurements, samples were brought to room temperature and centrifuged at 1500 g for 15 minutes; the clear top-phase of the sample was pipetted into appropriate test tubes and assayed using an enzyme immunoassay (EIA) protocol for salivary IL-6 normalized to total protein concentration (Interleukin-6 high sensitivity ELISA IBL®). Absorbance was performed on a spectro-photometer (Opsys MR^{TM}) using 450 nm as the primary wavelength. For IL-6 assays, intra-assay and interassay mean coefficients of variations were 4.0% and 6.0%, respectively.

For catecholamine measurements, all urine samples were collected into urine bottles with 0.5M hydrochloric acid as preservative and stored at −80° before analysis. Total norepinephrine, epinephrine, and dopamine were assessed by high performance liquid chromatography in reverse phase with amperometric electrochemical detection after hydrolysis (30 min at 100°C, at a pH of 0.8–1) and extraction by ionic exchange on the Recipe column (25). For catecholamine assays, the following intra-assay mean coefficients of variations (0.98% for norepinephrine, 1.73 for epinephrine, 1.94 for dopamine) and interassay mean coefficients of variations (4.3% for norepinephrine, 5.8% for epinephrine, and 5.6% for dopamine) were measured. Data are expressed as the ratio to urinary creatinine assessed using Jaffe's method. Total urinary testosterone was assessed by liquid chromatography tandem mass spectrometry and normalized to creatinine levels (every 3 h-period) during the waking period from 10:00 to 19:00. After acidic hydrolysis, testosterone was extracted by organic solvent (hexane and ethylacetate) and dry extract reconstituted in mobile phase and analyzed in mass spectrometry (intra-assay and interassay mean coefficients of variations were 6.5% and 8.0%, respectively). Data are expressed as the ratio to urinary creatinine assessed using Jaffe's method.

Statistics

For catecholamine and IL-6 analyses, within-session comparisons were first done by between-session comparisons to assess the effects of napping.

Within-session comparisons

The effects of sleep restriction/ad libitum sleep recovery were evaluated by two-way repeated measure ANOVA with a between-subject factor of sleep condition (control and sleep restriction/control and sleep recovery for "sleep restriction" and "sleep restriction + nap" sessions) and time as within-subject factor (10:00–13:00, 13:00–16:00, 16:00–19:00 for catecholamine analyses and 10:00, 13:00, 16:00, 19:00 for IL-6 analyses) completed by a pairwise comparisons post hoc test (Student-Newman-Keuls' test). Values were averaged across the day period when the time × condition interaction was not statistically significant.

Between-session comparisons

To test the effects of napping, comparisons between the different sleep restriction conditions were performed using normalized delta scores: (restriction − baseline)/baseline in "sleep restriction" session; (restriction + nap − baseline)/baseline) in "sleep restriction + nap" session. The normalized changes from baseline were then evaluated by a two-way repeated measure ANOVA with a between-subject factor of sleep condition (sleep restriction, sleep restriction + nap) and time as the within-subject factor (10:00–13:00, 13:00–16:00, 16:00–19:00 for catecholamine analyses and 10:00, 13:00, 16:00, 19:00 for IL-6 analyses) completed by a pairwise comparisons post hoc test (Student-Newman-Keuls' test).

For sleep analyses, comparisons among sessions were performed for each sleep parametes using a one way repeated measure ANOVA completed by a pairwise comparisons post hoc test (Student-Newman-Keuls' test).

Data were analyzed using the SigmaStat® 3.5 software (Systat®). The data showed a normal distribution (Shapiro-Wilk normality test). Values are expressed as mean values (SEM). A probability level of $P < .05$ was considered as statistically significant.

Results

Sleep architecture

There were no significant differences in sleep stage durations between baseline and sleep-restricted nights in the "sleep restriction" and the "sleep restriction + nap" sessions, indicating good experimental reproducibility (Table 1). Analysis of sleep recordings showed that there was significantly more slow-wave sleep (SWS) during the afternoon nap (in a more favorable circadian period) than the morning nap and shorter sleep latency [$F(1, 10) = 5.04$, $P = .04$, and $F(1, 10) = 6.32$, $P = .03$, respectively].

Table 1. Sleep Architecture and Variables in "Sleep Restriction" and "Sleep Restriction + Nap" Sessions

	"Sleep Restriction" Session			"Sleep Restriction + Naps" Session				
	Control	Restriction	Recovery ad Libitum	Control	Restriction	Nap 09:30	Nap 15:30	Recovery ad Libitum
Stage 1 (min)	15.3 ± 6.9	3.9 ± 1.6	37.0 ± 27.3	18.9 ± 17.1	4.3 ± 3.1	0.9 ± 0.9	0.3 ± 0.5	25.8 ± 21.7
Stage 2 (min)	191.0 ± 17.1	37.9 ± 8.6	316.9 ± 28.2	214.4 ± 40.1	38.2 ± 10.4	15.2 ± 5.8	12.9 ± 4.3	312.5 ± 36.5
SWS (min)	88.5 ± 16.3	63.1 ± 10.4	168.3 ± 8.4	87.2 ± 18.1	56.7 ± 9.1	11.5 ± 6.4	17.0[a] ± 3.5	144.3[b] ± 5.1
REM (min)	138.7 ± 22.3	16.1 ± 8.1	187.9 ± 29.4	115.3 ± 22.2	15.9 ± 6.9	3.2 ± 5.3	0.3 ± 0.6	196.6 ± 27.3
Total sleep time (min)	431.8 ± 22.9	117.5 ± 7.5	708.3 ± 21.0	436.2 ± 27.6	113.4 ± 8.1	30.0 ± 0.0	30.0 ± 0.0	681.1 ± 32.3
Sleep efficiency (%)	91.4 ± 3.0	97.9 ± 6.3	91.2 ± 2.8	90.9 ± 5.7	94.4 ± 6.7	n/a	n/a	90.3 ± 2.8
Sleep latency (min)	18.1 ± 6.7	4.2 ± 1.4	24.1 ± 6.9	12.7 ± 4.2	6.1 ± 4.7	8.2 ± 4.2	4.9 ± 2.3	33.2 ± 17.6

There were no significant differences in any sleep parameters during baseline and sleep-restricted nights between the "sleep restriction" and "sleep restriction + nap" sessions, indicating good experimental reproducibility. Mean ± SEM.

[a] Indicates significant differences vs Nap 9h30.

[b] Indicates significant differences vs recovery ad libitum "sleep restriction" session. Stage 1 and stage 2 sleep define light sleep and REM and SWS are abbreviations for slow-wave sleep and rapid eye movement sleep, respectively.

Daytime napping did not increase sleep latency, but was associated with a reduced amount of SWS [F (1, 10) = 9.72, $P = .01$] and a trend to decreased total sleep time [F (1, 10) = 3.4, $P = .06$] during the subsequent unlimited recovery night.

Biomarker within-session comparisons

Urinary catecholamines after sleep restriction without or with napping

The day after the sleep restricted night, there was a 2.5-fold increase in norepinephrine levels in the afternoon (16:00–19:00) compared to the same period during the control day [F (1,18) = 6.3 $P = .03$; $P = .003$ vs same time control condition, post hoc test; Figure 1A]. However, after a 30-minute nap in the morning and the afternoon, these changes in norepinephrine levels were not present [F (1, 18) = 0.02, $P = .88$; Figure 1B].

There was no time effect or significant interaction between time and sleep condition in the "sleep restriction" or the "sleep restriction + naps" sessions [(F 2, 18) = 2.4 $P = .12$ and (F (2,18) = 2.5 $P = .12$, respectively and F (2,18) = 0.38, $P = .68$ and F (2,18) = 0.49, $P = .62$, respectively]. Hence, values were averaged across the day period and the norepinephrine concentrations per day were also higher in the "sleep restriction" session ($P = .03$ vs control condition, post hoc test Figure 2A), but not in the "sleep restriction + nap" session (Figure 2B) relative to the respective control day.

Although similar variations were also observed for epinephrine, these were not statistically significant (Figure 1, C and D).

Dopamine levels were not affected by sleep restriction at any time point tested (Figure 1, E and F).

In an additional assay for urinary testosterone, we found no significant changes during the day after the sleep restricted night compared to the same periods during the control day [(F (1,18) = 1.2 $P = .3$ vs same time control condition (see Supplemental Table 1)].

Salivary interleukin-6 after sleep restriction without or with napping

Il-6 levels were significantly lower at 10:00 and 19:00 the day after the sleep restricted night compared to the same time control condition [F (1, 29) = 7.0, $P = .02$; $P =$ 0. 01 and 0.05 vs same time control condition, respectively, post hoc test Figure 3A]. These decreases in IL-6 levels were no longer observed at 10:00 and 19:00 when the subjects had the opportunity to nap for 30 minutes at 09:30 and 15:30 [F (1,29) = 0.46, $P = .51$; Figure 3B].

There was no time effect or significant interaction between time and sleep condition in the "sleep restriction" or the "sleep restriction + naps" sessions [F (3, 29) = 0.68, $P = .57$ and F (3, 29) = 1.86, $P = .16$, respectively, and F (3, 29) = 0.46, $P = .51$ and F (3, 29) = 1.1, $P = .38$, respectively]. Il-6 values were then averaged across the day period and the IL-6 concentrations per day also showed reduced levels in the "sleep restriction" session. ($P = .03$ vs control condition, post hoc test Figure 3C) but not in the "sleep restriction + nap" session (Figure 3D) relative to the respective same time control day values.

Biomarkers after ad libitum sleep recovery

During the recovery day in the "sleep restriction session", there was increased release of afternoon epinephrine [F (1,18) = 7.5, $P = .02$; $P = .02$ (13:00–16:00) and 0.004 (16:00–19:00) vs same time control condition, post hoc test; Table 2]. In contrast, there were no significant changes in urinary epinephrine levels in the "sleep restriction + nap" session [F (1, 18) = 0.81 $P = .39$; Table 2]. Similarly, dopamine levels were increased at 16:00–19:00 in the "sleep restriction" session but not "sleep restriction + nap" session [F (1, 18) = 4.9 $P = .03$; $P = .01$ vs same time

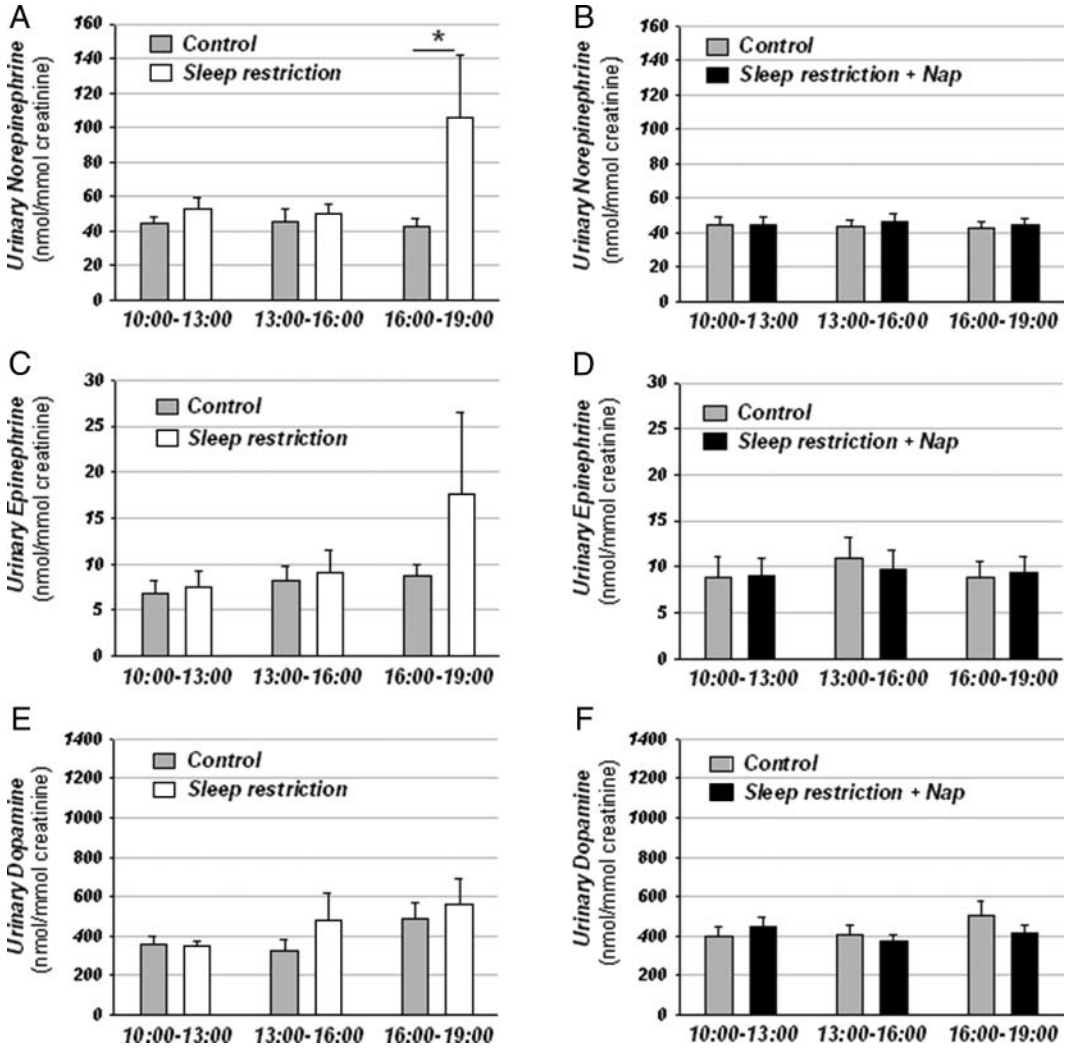

Figure 1. Urinary catecholamines (norepinephrine, epinephrine, and dopamine) normalized to creatinine levels were assessed every 3 h during the waking period from 10:00 to 19:00. Control and postsleep restriction values are showed in "sleep restriction" (A, C, and E) and "sleep restriction + nap" (B, D, and F) sessions. Mean ± SEM; * significant vs respective same time control condition.

control condition, post hoc test and F (1, 18) = 1.8 $P = .2$, respectively; Table 2].

At all-time points tested, there were no statistically significant changes in salivary IL-6 levels compared to the same time control conditions (Table 2).

Biomarker between-session comparisons

To assess the effects of napping, normalized changes from baseline in urinary catecholamine and IL-6 levels during the sleep restricted day were compared between the "sleep restriction" and the "sleep restriction + nap" sessions.

The effects of sleep restriction on norepinephrine concentrations varied according to the sleep restriction condition [F (1, 18) = 7.42, $P = .02$]. Pairwise between-session comparisons showed a significant difference between the "sleep restriction" and the "sleep restriction + nap" session, indicating a stress-releasing effect at the end of the afternoon (16:00–19:00) in the participants who napped ($P = .004$, post hoc test; Figure 4A).

The effects of sleep restriction on the levels of epinephrine and dopamine at all-time points tested did not change according to the sleep restriction condition [F (1, 18) = 0.14, $P = .71$; F (1, 18) =0.53, $P = .537$ Figure 4, B and C].

The effects of sleep loss on IL-6 levels changed depending on the sleep restriction condition [F (1, 30) = 8, 8, $P = .01$]. There was a significant difference between the "sleep restriction" and the "sleep restriction + nap" session at 10:00, 13:00, and 19:00 time points indicating a normalizing effect of the nap on the IL-6 changes induced by sleep restriction ($P < .001$, $P = .02$, $P = .003$, respectively, post hoc test; Figure 4D).

Discussion

The present investigation showed that daytime naps for no more longer than 30 minutes after a night with only 2 h of

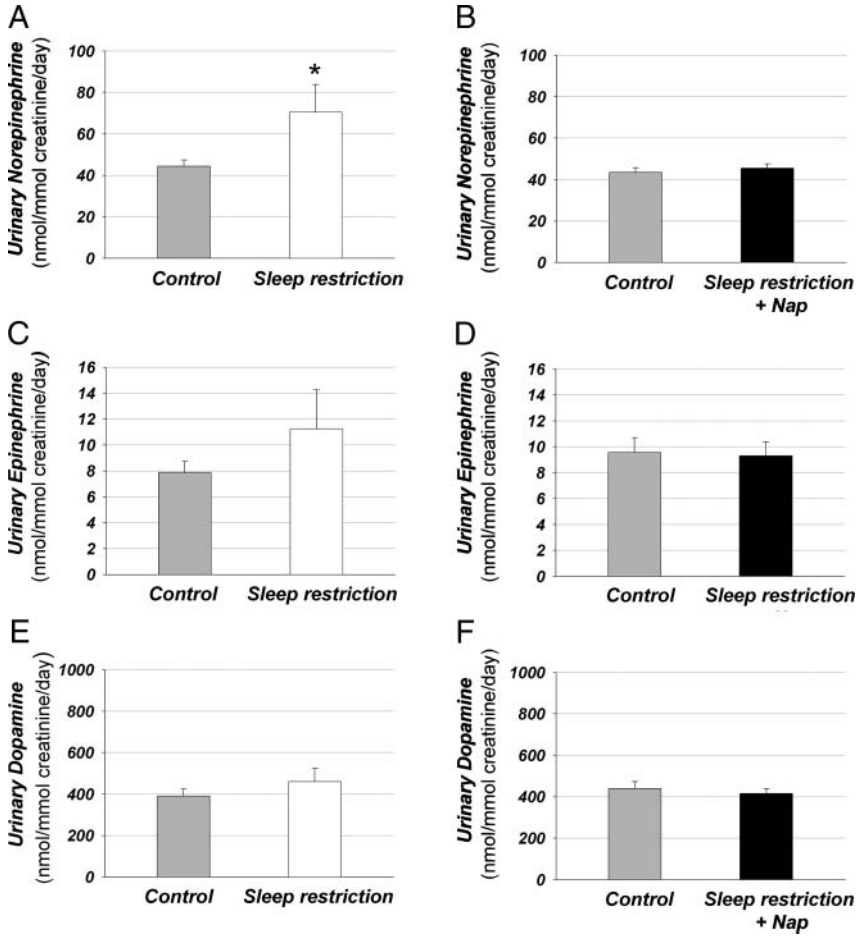

Figure 2. Urinary catecholamines (norepinephrine, epinephrine, and dopamine) per day, normalized to creatinine levels, are shown. Values were averaged across the day period when the time × condition interaction was not statistically significant in "sleep restriction" (A, C, and E) and "sleep restriction + nap" (B, D, and F) sessions. Mean ± SEM; * significant vs respective same time control condition.

sleep restores urinary catecholamine and salivary IL-6 levels altered by sleep loss to baseline levels. To our knowledge, no experimental data have previously reported stress-releasing and immune effects of a multinap protocol in continuously PSG-monitored subjects. We acknowledge that this study was performed in a relatively small sample size of subjects, although their healthy status is expected to reduce intersubject variability. Interestingly, only a few studies have observed stress and immune responses following a 30 minute nap (13) or longer 2 h naps (14, 26). These effects and the nature of the stage(s) of sleep involved (SWS?) need to be further elucidated. Moreover, whether these napping-dependant effects could be detectable noninvasively in biofluids, such as urine and saliva, was not known. This issue is of importance to identify potential tools for evaluating the effects of practical napping strategies in chronically sleep-deprived populations, such as night and shift workers.

We reported here that sleep restriction enhanced urinary catecholamine levels ie, norepinephrine and that nap episodes limited this effect. Sleep and its SWS component during the night are believed to contribute to a reduced release of major mediators, cortisol and catecholamines, of the hypothalamic-pituitary adrenal and sympathoadrenal stress systems (27–9). Accordingly, a stress-releasing effect is induced by napping as indicated by the decrease in cortisol levels observed during a 2 h midday nap or immediately after a shorter 30 minute midday nap with half of both the naps consisting of SWS (13, 14). Here, we additionally showed that napping could induce a stress-releasing effect as measured by urinary norepinephrine.

However, we need to further evaluate the precise timing of this stress-releasing effect and its action on the immune system.

We also showed that salivary IL-6 levels were changed the day after the nocturnal partial sleep deprivation but normalized after napping. When focusing on serum IL-6 data after a single night of restricted sleep as in our design, the suppression of sleep during the first half-part of the night showed that the normal evening increase in IL-6 levels in healthy men was delayed until sleep at 03:00. The same early-night sleep deprivation protocol further indicated that women and men both showed a significant increase in the production of IL-6 in stimulated peripheral blood monocytes in the morning immediately after sleep restriction, whereas production of these cytokines during the early and late evening was increased in women, but decreased in men suggesting differential sex effects of sleep restriction on IL-6 (30). Similarly, we found that in men, salivary IL-6 levels were decreased in the early evening.

The only previous study to our knowledge on salivary IL-6 after sleep loss reported that 30 h of sustained waking was required to increase IL-6 levels measured at 14:00 the day after the total sleep-deprived night (31). In our experiment, we did not measure the 14:00 time point and applied a partial sleep deprivation procedure closer to the natural environment of sleep-deprived individuals. Serum IL-6 levels during the day after a total sleep deprived night have been shown to be increased or decreased in healthy men and women (14, 32). Although the reasons for these

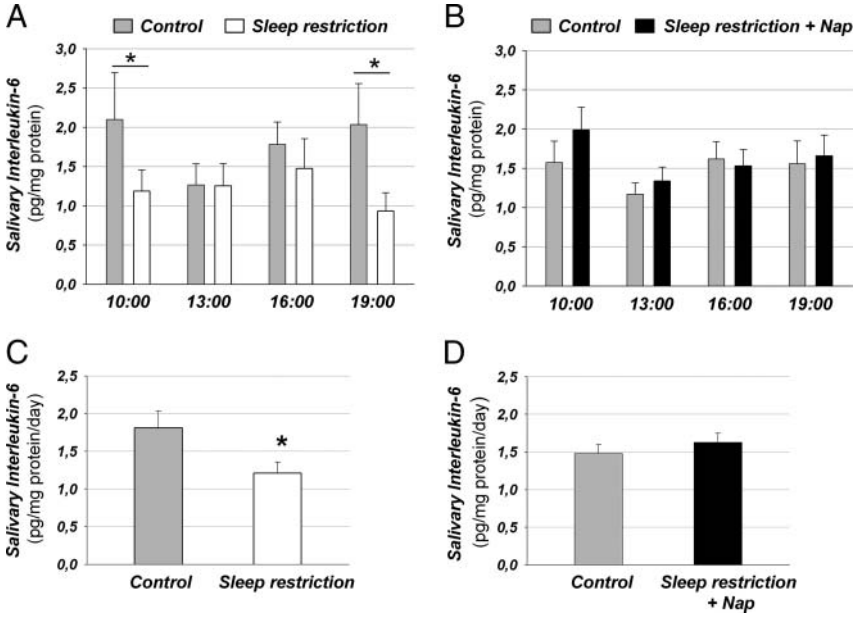

Figure 3. Salivary interleukin-6 normalized to total protein was assessed every 2 h, including immediately after the naps and urine was sampled every 3 h during the waking period from 10:00 to 19:00. Control and postsleep restriction values are showed in "sleep restriction" (A) and "sleep restriction + nap" (B) sessions. Values were averaged across the day period when the time x condition interaction was not statistically significant in "sleep restriction" (C) and "sleep restriction + nap" (D) sessions. Mean ± SEM; * significant vs respective same time control condition.

apparent discrepancies remain unclear, the fact that intravenous (IV) catheters used for repetitive blood sampling can increase local IL-6 production is a potential contributing factor, as is the mix of men and women in the same group (30, 33). Salivary measurements have the advantages of not inducing such methodological confounders.

Two previous studies indicated that following total sleep deprivation, long 2 h-nap episodes reduced the effects of total sleep deprivation on serum IL-6 levels (14, 25). Reduced IL-6 concentrations were observed for several hours following a 2 h midday nap between 14:00 and 16:00 compared to the sleep-deprived values (14).

After one night of recovery sleep, Il-6 levels were not altered compared to control conditions at all time-points tested in our study. Similar findings have been reported in blood levels after recovery sleep (samples analyzed after two nights of recovery sleep) following a week of chronic partial sleep deprivation (34).

In summary, IL-6 regulation appears complex and dependent on the biofluid tested, the measurement time and the sleep deprivation and recovery procedure. However, in a realistic situation of partial sleep loss, naps of no longer than 30 minutes reverse the changes observed after sleep loss.

What are the potential mechanism(s) that can lead to modified markers of the stress and immune systems after sleep deprivation? Sleep loss counterbalanced by sleep recovery episodes, such as naps, produced significant changes in stress and immune markers, indicating sleep-dependent interactions between the central nervous system (CNS), and the neuroendocrine and immune functions.

We previously hypothesized that an increase in catecholamine levels during a sleep restricted night may contribute to the increased neutrophil counts we observed in the morning (13, 35).

Using exactly the same sleep restriction procedure as in the current study, we showed that there were no significant changes in salivary daytime cortisol suggesting that its anti-inflammatory properties are not involved in the IL-6 changes (13). This also suggests that partial sleep deprivation when limited to one night does not markedly affect cortisol level during the morning and the afternoon following the night of sleep loss and suggests that the hypothalamic-pituitary-adrenal (HPA) axis is not highly solicited with this degree and period of sleep deprivation during the day period (13). An additional point in favor of this hypothesis is a similar absence of urinary total testosterone changes measured during the day after sleep restriction. Indeed, in the bidirectional interactions between the HPA axis and the hypothalamic-pituitary-gonadal axis, activation of the HPA stress system could have potentially resulted in reduced testosterone release and/or changed testosterone levels could have modulate the HPA axis response (36). Most reports on the effect of sleep loss on testosterone levels mainly found that total sleep deprivation and several consecutive days of sleep curtailment, a more chronic and stressful procedure than a single night of partial sleep deprivation, were associated with reduced testosterone levels (37–39). A recent report also suggests that the effects of sleep deprivation on testosterone levels depend on the time of the sleep restriction period: sleep deprivation during the second part of the night (sleeping from 22:30 to 03:30) reduces morning testosterone levels while sleep deprivation in the first part of the night (sleeping from 02:45 to 07:00) does not change testosterone levels (40). In our design, the sleep period was restricted to the middle part of the night (sleeping from 02:00 to 04:00) and similarly did not induce significant testosterone changes.°

Table 2. Urinary Catecholamines (Norepinephrine, Epinephrine and Dopamine) Normalized to Creatinine Levels (Every 3 h Period) and Salivary Interleukin-6 Normalized to Total Protein (Every 2 h) Were Assessed During the Waking Period From 10:00 to 19:00. Control and Post-Sleep Recovery Values are Shown for the "Sleep Restriction" and "Sleep Restriction + Nap" Sessions

	"Sleep Restriction" Session							
	Control				**Recovery**			
	10:00–13:00	**13:00–16:00**		**16:00–19:00**	**10:00–13:00**	**13:00–16:00**		**16:00–19:00**
Norepinephrine (nmol/mmol creatinine)	44.6 ± 3.7	45.7 ± 7.6		43.0 ± 3.9	41.6 ± 2.8	52.1 ± 4.0		68.4 ± 16.1
Epinephrine (nmol/mmol creatinine)	6.7 ± 1.5	8.2 ± 1.6		8.7 ± 1.3	6.5 ± 1.6	13.0 [a] ± 3.2		18.5[a] ± 4.8
Dopamine (nmol/mmol creatinine)	358.7 ± 37.4	327.9 ± 55.7		489.3 ± 77.4	324.6 ± 34.3	741.7 ± 202.7		1100.2[a] ± 335.6
	Control				**Recovery**			
	10:00	**13:00**	**16:00**	**19:00**	**10:00**	**13:00**	**16:00**	**19:00**
Interleukin-6 (pg/mg protein)	2.0 ± 0.6	1.3 ± 0.3	1.8 ± 0.3	2.0 ± 0.5	1.4 ± 0.3	1.4 ± 0.4	1.5 ± 0.3	1.9 ± 0.4
	"Sleep Restriction+ Nap" Session							
	Control				**Recovery**			
	10:00–13:00	**13:00–16:00**		**16:00–19:00**	**10:00–13:00**	**13:00–16:00**		**16:00–19:00**
Norepinephrine (nmol/mmol creatinine)	44.2 ± 5.1	43.7 ± 3.7		42.4 ± 4.0	41.0 ± 3.9	50.5 ± 11.8		64.5 ± 16.5
Epinephrine (nmol/mmol creatinine)	8.9 ± 2.1	11.0 ± 2.2		8.8 ± 1.7	6.8 ± 1.8	9.6 ± 1.9		13.9 ± 3.2
Dopamine (nmol/mmol creatinine)	400.3 ± 47.0	406.6 ± 45.7		507.2 ± 74.2	368.6 ± 61.0	473.6 ± 71.1		722.2 ± 126.4
	Control				**Recovery**			
	10:00	**13:00**	**16:00**	**19:00**	**10:00**	**13:00**	**16:00**	**19:00**
Interleukin-6 (pg/mg protein)	1.6 ± 0.3	1.2 ± 0.1	1.6 ± 0.2	1.6 ± 0.3	1.5 ± 0.3	1.2 ± 0.2	1.6 ± 0.3	1.4 ± 0.2

Mean ± SEM.

[a] Significant vs respective same time control condition.

However, in IL-6 potential regulating mechanism(s), norepinephrine has been shown to inhibit the stimulated IL-6 and TNF-α production by human whole blood and human monocytes in a concentration-dependent manner (41, 42). This mechanism may account for the reduced salivary IL-6 levels at the end of the afternoon. We also observed increased levels of epinephrine and dopamine after the unlimited recovery night. Although these effects are not clearly understood, sleep has been described previously as enhancing the changes in neuroendocrine activity, including an increased release of dopamine (43).

Immune alterations may also affect sleep architecture (44). Previous results in healthy adults (24–61 years old) indicated that increases in stimulated monocyte production of IL-6 in the late evening (23h00) were associated with decreased SWS duration (45). Similarly, we found that lower salivary IL-6 levels at the end of the afternoon after sleep restriction were associated with increased SWS during the subsequent recovery night. However, the potential relative role of IL-6 on SWS regulation needs to be discriminated from the major effect of SWS homeostatic pressure.

In a more clinical perspective, the potential mechanism(s) underlying the relationships between mood and sleep in sleep-deprived depressed patients have been suggested to concern the, serotoninergic system (46). However, alternative mechanisms could be proposed. Davis and colleagues (47) measured after sleep deprivation increased levels of serotonin but also, tryptophan, and taurine which could explain the antidepressive effect of acute sleep deprivation. Alternatively, increase of norepinephrine and/or decrease of IL6 may be part of factors for improving mood as reported here as an effect of sleep loss. Interestingly current literature provides support to the view that on the one hand antide-

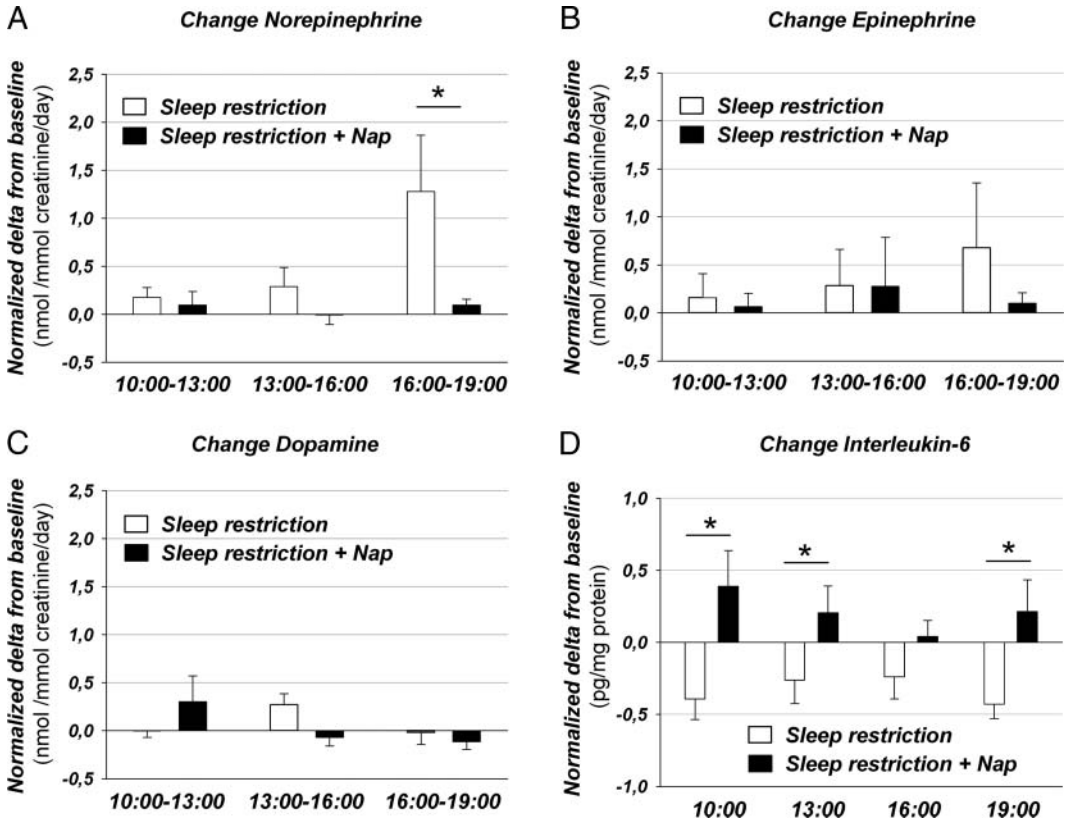

Figure 4. To test the effects of napping, comparisons between the different sleep restriction conditions ("sleep restriction" session and "sleep restriction + nap" session) were performed using normalized delta scores for each sleep restriction condition relative to the respective baseline. Changes are shown for norepinephrine (A), epinephrine (B), dopamine (C), and interleukin-6 (D). Mean ± SEM; * significant vs same time control condition.

pressants that inhibit the reuptake of norepinephrine are used as first-line treatments against depression, and on the other hand that depression is positively associated with a proinflammatory status and higher levels of proinflammatory cytokines such as IL-6 or TNF-α (48, 49).

To conclude, the 2-h sleep duration that we studied is close to the situation of extended work shifts experienced by workers such as interns during residency training (50).

It has been shown that, after a 30 h extended work shift [sleep duration 0.3 (0.0–1.5) h], young internal medicine residents (mean age 29 years) had increased blood levels of norepinephrine and decreased flow-mediated vasodilatation compared to the same residents after a nonextended work shift (51). In addition, a naturalistic actigraphy investigation in 96 middle-aged subjects reported that lower sleep efficiency was associated with increased 24-h urinary levels of norepinephrine (52). Norepinephrine levels are described as reflecting overall sympathetic activity, particularly solicited during periods of altered sleep duration and quality.

Our present results and previous data suggest that the inhibition of cortisol and catecholamine release by a 30-minute nap is critical for recovery from stress and immune alterations (12). Although IL-6 is usually considered as a proinflammatory cytokine, some data have indicated possible anti-inflammatory properties by inhibiting the expressions of proinflammatory cytokines, making the interpretations of the effects of sleep restriction on IL-6 complex (53). To better understand the physiological mechanism(s) underlying the links, between sleep deprivation and recovery, and these immune and inflammatory changes, in addition to IL-6 assessed here, the investigation of others immune regulatory cytokines (proinflammatory, eg, TNF-α, IL-1-ß, IL-17) and anti-inflammatory, eg, interferon (IFN)-α, transforming growth factor (TGF-ß), would be required in further studies. The effects of circadian disruption and sleep restriction on these systems need also to be investigated in relevant populations, such as night and shift workers (sleeping 1–2 h less per 24 h than day workers), with noninvasive biomarkers from laboratory-based pilot studies (54).

Napping as a countermeasure to sleep restriction could, in addition to benefits on alertness, improve neuroendocrine stress and immune recovery with a potential prophylactic long-term effect on cardiovascular health.

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